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PARATYPHOID BACILLI FROM CHICKS

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During several years past we have experienced many serious losses among chicks, wherein the lesions have been fairly constant, and the preliminary bacteriologic examinations have shown the presence of paratyphoid bacteria. Since there was considerable doubt as to the exact nature of the organisms concerned, it was deemed advisable to undertake an intensive study of organisms from a number of outbreaks. Considerable work has been done by others in connection with the etiology of bacillary white diarrhea in chicks, but for the most part such work has been with old laboratory strains of *Bact. pullorum* and related organisms.

Our study comprises 21 strains of paratyphoids freshly isolated from 21 outbreaks of highly destructive disease among chicks. For comparison there are included 2 strains from ovaries of mature hens; one official strain each of *Bact. gallinarum*, *Bact. pullorum*, *B. enteritidis*, *B. paratyphosus* A, *B. paratyphosus* B, and *B. typhosus*. The chicks attacked in these outbreaks were usually from 2 to 4 days old. The lesions most constantly found in the chicks were slight enlargement of the liver together with petechiae and necrotic foci. These liver lesions were found in at least 90% of the chicks from which paratyphoid bacteria were isolated, and were rarely found in chicks from which paratyphoids could not be isolated. Pneumonia was found in about 70% of the affected chicks. Quite frequently yellowish, friable nodules were found in the lungs. Occasionally these nodules were found in organs other than the lungs. Abscesses in the cecums were found in about 8% of the cases.

Bacteriologic Study.—No new technic has been introduced in this study, but certain methods of recent origin were used, and it is believed that interesting information, relative particularly to *Bact. pullorum* and *Bact. gallinarum*, is first reported herein on recently isolated strains. Control tests were made in all mediums on authentic strains of *Bact. gallinarum* G 1, *Bact. pullorum* P 1, *B. paratyphosus* A-A 97, *B. paratyphosus* B-B 96, *B. enteritidis* E 117, and *B. typhosus* T 114.

Morphology.—All of the 29 strains were gram-negative, and were of a size and form common to the paratyphoid bacilli. All of our strains, except strain P 14, were identical with the type strain of *Bact. pullorum* P 1, and commonly exhibited a tendency toward bipolar and peripheral staining.

Motility.—Control strains A 97, B 96, E 117, and T 114, and our strain P 14, were actively motile; control strains G 1, P 1, and our other 22 strains were nonmotile. The organisms of strain P 14 appeared to bear a single polar flagellum, although some cells seemed to have a second one attached at the same pole. Flagella could not be demonstrated on any of our other strains. Typical peritrichal flagella were readily demonstrable on the control strains A 97, B 96, T 114, and E 117, but were not demonstrable on the control strains G 1 and P 1.

Cultural Characteristics.—Twenty-two of our strains may be grouped for discussion, but strain P 14 apparently represents a new type, and will be treated separately.

No indol reaction could be demonstrated in any of our 23 strains, nor in any of the control strains.

All of our 23 strains produced H_2S in lead acetate agar. A positive reaction was also obtained for all control strains except *B. paratyphosus* A-A 97.

All strains, including control strains, gave an initial acidity in litmus milk which lasted over a varying period, returning to neutral then alkaline in the following order: *B. enteritidis* E 117 alkaline at 3 days; *B. paratyphosus* B-B 96 at 8 days; *Bact. gallinarum* G 1 at 13 days; *B. typhosus* T 114 at 25 days; *B. paratyphosus* A-A 97 at 35 days; *Bact. pullorum* P 1 at 35 days.

Of our strains, P 14 became alkaline after 3 days. The remaining 22 strains retained their acidity for at least 70 days. At 72 days one strain was perceptibly alkaline; 9 strains became alkaline between 72 and 95 days; and the remaining strains were neutral or acid at 96 days.

Milk cultures of control strains became translucent and opalescent in the following order: *B. enteritidis* E 117, *B. paratyphosus* B-B 96, and *B. typhosus* T 114 at 53 days; *Bact. gallinarum* G 1 at 90 days; *B. paratyphosus* A-A 97 at 104 days; *Bact. pullorum* P 1 remained opaque at 107 days. Of our strains, P 14 became translucent and opalescent at 72 days, and the remaining 22 strains were still opaque at 107 days.

Considerable work has been done on the subject of fermentation of carbohydrates in the effort to differentiate *Bact. pullorum* from *Bact. gallinarum*. The method employed in these studies was, for the most part¹ based on titration at varying periods of extract or infusion broth cultures containing 1% of the desired carbohydrate. Krumwiede and Kohn² substituted the use of the Andrade indicator in the sugar broth for titration.

We have found that the use of 1% sugar-serum-water plus the Andrade indicator, as advocated by Krumwiede, Kohn, and Valentine,³ possesses certain advantages over titration in that immediate and frequent observations may be made; gas production is readily detected; the slightest initial acidity may be noted; and reducing ability may be distinguished by the decolorization of the coagulated serum. Another indicator, "C-R,"⁴ was tried, but it possessed no advantage over the Andrade indicator.

¹ Hadley, P., Rhode Island Bull., 178, 1918; Smith, T., and Tenbroeck, C., Jour. Med. Res., 1914, 26, p. 547; Goldberg, S. A.: Jour. Amer. Vet. Med. Assoc., 1917, 51, p. 203.

² Jour. Med. Res., 1917, 31, p. 511.

³ Jour. Med. Res., 1918, 38, p. 89.

⁴ Bronfenbrenner, Davis, and Morishima, Jour. Med. Res., 1919, 39, p. 346.

As a preliminary test for native sugar in the serum, tubes of serum-water (1 part serum, 4 parts distilled water, plus 1% of the Andrade indicator) were prepared without the addition of any sugar. These were inoculated with the 6 control strains and incubated for 3 days. No trace of acidity was perceptible at any time, and as all tests were made in the same lot of serum-water, fermentation subsequent to the addition of a known sugar could therefore be reasonably ascribed only to the presence of such sugar.

Control strains G 1, P 1, and T 114, and 12 of our 23 strains gave acid, coagulation, and no gas in glucose; control strains B 96, A 97, E 117, and 11 of our strains gave acid, coagulation, and varying amounts of gas; control strains B 96 and E 117, and our strain P 14 completely decolorized the indicator. The test was immediately repeated on all strains which produced no gas, and identical results were obtained.

All strains were then tested in fermentation tubes of 1% glucose infusion broth (rendered sugar-free by *B. coli*) plus Andrade indicator. Control strains G 1, P 1, T 114, and 5 of our strains gave acid with no gas. All other strains gave acid and gas in amounts varying from a bubble to 20%. In comparing the results of this test in broth with the above tests in serum-water we find that 2 of our strains did not produce gas in glucose broth but did in serum-water; 8 of our strains produced gas in glucose broth but did not in serum-water; control strains G 1, P 1, T 114, and 3 of our strains were constantly negative for gas.

All strains, including controls, were negative for acid and gas in lactose infusion broth. In lactose serum-water all strains, including controls, gave a slight but distinct acid reaction at 4 to 8 hours. At 18 hours B 96, A 97, and E 117 were colorless, while the reaction persisted in all of the others without intensifying for 24 to 48 hours. In no case was the acidity sufficient to coagulate the serum. This test was repeated with identical results. Whether this slight reaction represented an actual fermentation of lactose, or of a product of hydrolysis due to sterilization is not known.

The results with dextrin were unsatisfactory; control strains G 1, A 97, B 96, T 114, and E 117, all produced undoubted acidity, but not of sufficient strength to coagulate the serum. In the absence of coagulation the determination of gas was also unsatisfactory. However, *Bact. pullorum* P 1, commonly described as unable to ferment dextrin, and all of our strains produced slight but distinct acidity. The factor of hydrolysis, however, may again have entered into these results.

All strains were negative on two tests in saccharose and salicin. Control strain B 96 and our strain P 14 produced acid, gas, and coagulation in inosite, and decolorized the indicator, thus conforming to the classification of a true *B. paratyphosus* B. *B. enteritidis* E 117 and all other strains gave an entirely negative reaction. With dulcitate control strains B 96, E 117, and our strain P 14 gave acid, gas, and coagulation, and decolorized the indicator. Control strain A 97 gave acid, gas, and coagulation without decolorization; control strain G 1 gave acid, coagulation, and some decolorization, but no gas; control strain P 1, T 114, and all of our strains, except P 14, showed no fermentation.

Control strains B 96, E 117, and our strain P 14 gave acid, gas, coagulation, and complete decolorization in xylose; control strain T 114 gave acid and coagulation, but no gas nor decolorization; control strains A 97, G 1, P 1, and all of our strains, other than P 14, were uniformly negative.

Control strains B 96, E 117, and our strain P 14, gave acid, gas, coagulation, and decolorization in mannite; control strain A 97 gave acid, gas, and coagulation, but no decolorization; control strains G 1, P 1, and T 114 gave acid

and coagulation, but no gas nor decolorization. Of our strains, 4 gave acid and coagulation, but no gas nor decolorization; all others gave acid, gas, and coagulation, but no decolorization.

In comparing the fermentation of mannite with that of glucose we find that, of the 12 strains which did not produce gas in glucose, 8 produced gas in mannite; 4 strains were negative for gas in both sugars.

Control strains B 96, E 117, and our strain P 14 produced acid, gas, coagulation in levulose, and decolorized the indicator; control strain A 97, and 10 of our strains, other than P 14, produced acid, gas, and coagulation, but no decolorization.

In galactose the control strains, and our strain P 14, behaved exactly as in levulose; on the first test one of our strains, other than P 14, produced acid, gas, and coagulation, with no decolorization; 21 strains produced acid and coagulation, but no gas nor decolorization. This test was immediately repeated, and gas was obtained from 3 strains which were negative on first test. Two weeks later the test was again repeated and 3 strains which produced gas on the second test showed gas, while the strain which formed gas on the first test failed to show gas on the third test.

In arabinose control strains B 96, A 97, E 117, G 1, P 1, and our strain P 14 behaved as in levulose and galactose; control strain T 114 produced only slight acid, with no gas nor coagulation; 3 of our strains produced acid, gas, and coagulation, and the other 19 produced acid and coagulation but no gas.

In view of the statements of other investigators^{1,2} regarding the differentiation of *Bact. gallinarum* from *Bact. pullorum* on the grounds of ability or inability to ferment maltose, we were surprised to find a perceptible acidity at 4 hours in all tubes of maltose serum-water inoculated with our 23 strains. At 18 hours the medium was coagulated, and 16 strains had produced gas. The test was immediately repeated with identical results.

The test was again repeated, this time in fermentation tubes of infusion broth, previously rendered sugar-free by a strain of *B. coli*. One per cent. maltose and 1% Andrade indicator were added to the medium. Our results conformed to those of other investigators; control strains G 1 and T 114 developed strong acid but no gas; control strains A 97, B 96, E 117, and our strain P 14 developed acid and gas; control strain P 1, and all of our strains, other than P 14, gave no evidence of acid nor gas on 5 days' incubation.

After consideration of this conflicting evidence the test was again repeated in serum-water, using the "C-R" indicator. Our first findings were confirmed; this time all of our strains but one formed gas. For several strains this was the first evidence of gas production in any medium.

The factor of hydrolysis must again be considered, and we do not attempt to explain why maltose infusion broth and maltose serum-water, autoclaved alike at 15 pounds for 15 minutes, should give such contradictory results. The serum-water for the second test was sterilized in an Arnold sterilizer in an attempt to avoid hydrolysis. Whether or not the intermittent method offers any advantage over autoclaving, the results on inoculation were the same. Krumwiede and Kohn² have noted fermentation in maltose broth inoculated with strains of *Bact. pullorum* and incubated over a period of 16 to 40 days. They, too, consider the extent to which hydrolysis may have influenced their observations.

It has been observed by Hadley¹ that some organisms produce gas in sugar infusion broth but do not produce gas in sugar extract broth, and that acid production is similarly affected. It is possible that serum-water, notably an ideal medium, may in the same way influence fermentation by organisms that possess a low avidity for the given sugar.

Agglutination Tests.—The agglutinative affinities of this group of organisms have been so thoroughly studied by Krumwiede and Kohn, Smith and Tenbroeck, Hadley, and others, that we have used the test not in an attempt to differentiate, but to group the organisms included in our study. We have found that, of our 23 strains, 20 strains from chicks and the two strains from diseased ovaries of hens are apparently identical with the type strain of *Bact. pullorum* P 1. While cross agglutination with *Bact. gallinarum* serum and antigen was observed to a high titer, there was a well-marked distinction between our strains and the control strain of *Bact. gallinarum* G 1.

Strain P 14, which gave cultural reactions identical with those of a true *B. paratyphosus* B, was peculiarly distinct agglutinatively from the control strain B 96, and is apparently a newly observed type.

SUMMARY

In this study of freshly isolated paratyphoids from chickens we have found 20 strains from chicks showing lesions of bacillary white diarrhea, and 2 strains from diseased ovaries of hens, to be identical with a type strain of *Bact. pullorum*. One new type was encountered which closely resembled the *B. paratyphosus* B type, but which was agglutinatively distinct, and appeared to have commonly only a single polar flagellum; some cells doubtfully displayed a second.

Constant lesions were associated with the presence of these organisms; enlargement of the liver, together with petechiae and necrotic foci in the liver, and pneumonia and yellow friable nodules in the lungs were characteristic. Abscesses in the cecum were found occasionally.

One strain isolated from a chick fermented the proper sugars but did not produce gas from any. All other strains produced gas from one or more sugars.

No correlation could be shown in gas production from various sugars; some strains produced gas from glucose and not from mannite; the behavior of others was exactly the reverse. Repeated tests were necessary to demonstrate gas production by some strains.

All of our strains and the control strain of *Bact. pullorum*, usually regarded as unable to ferment maltose, produced acid and gas in 1% maltose serum-water. Maltose infusion broth was not fermented.